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Nearly half of human breast cancers are dependent on estrogen for growth. How estrogens stimulate cell growth is not understood. Estrogens activate an intracellular protein, the estrogen receptor (ER), which acts together with certain receptor-interacting proteins to "switch on" some genes involved in cellular proliferation. The goal of this project is to identify and elucidate the roles of such receptor-interacting proteins in ER function. I have identified three proteins that interact with the ER using the yeast two-hybrid system, including the Vitamin D receptor-interacting protein 150 (DRIP150), subunit of a nuclear receptor coactivator complex. I am currently defining the effects of DRIP150 on ER transcriptional activity using a transient transfection system designed to monitor ER-dependent transcriptional activation.

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Table of Contents

Introduction

The estrogen receptor alpha (ER) is a ligand-dependent transcription factor, which is involved in activation of genes involved in cellular proliferation. Estrogen receptor activation has been shown to be a factor in the progression of nearly half of all human breast cancers. The mechanism by which ER interacts with cellular factors that mediate transcriptional activation is therefore an important target for breast cancer therapy and prevention which is in need of further characterization.

ER is a ligand-dependent transcription factor. In the absence of hormone, ER exists as an aporeceptor complex which, upon ligand binding, dissociates from the complex and binds as a dimer to estrogen response elements on the DNA, mediating transcriptional initiation of estrogen-responsive genes. Activation of these genes by ER is thought to be regulated by coactivator proteins. These factors interact with ER via two transcriptional activation domains: an N-terminal hormone-independent activation domain termed AF-1 and a C-terminal hormone-dependent activation domain termed AF-2. Recruitment of accessory proteins to these domains is responsible for regulation of transcriptional activation by ER.

The inactivation of ER is the currently the primary method of treatment for ER-positive breast cancers. The identification of ER associated coactivator proteins that affect ER function is therefore important in elucidating the molecular mechanisms involved in ER-positive breast cancer development and may become important targets for intervention and therapy. The purpose of this study is to isolate these factors using a yeast two-hybrid approach and to characterize their effects on ER transcriptional regulation.

Body

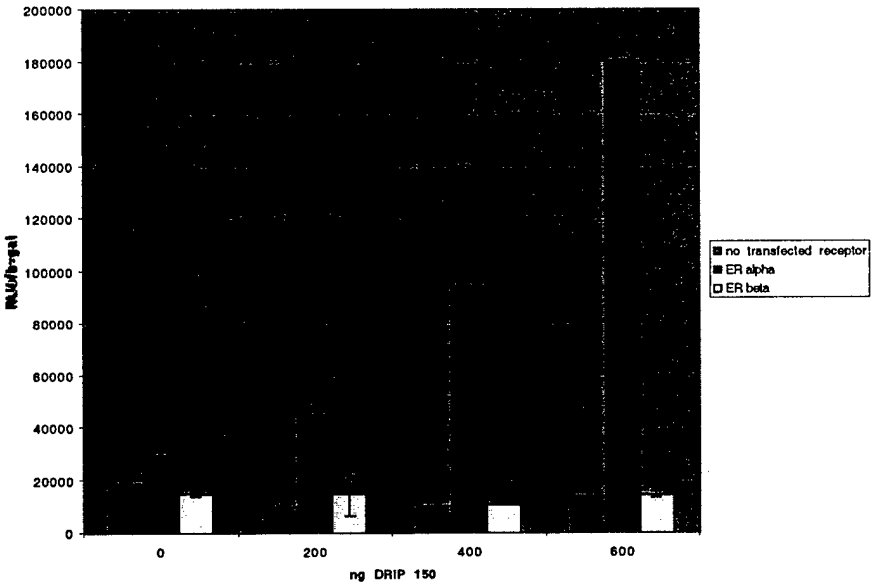
The purpose of this study is to isolate and characterize factors that regulate ER transcriptional activation through a yeast two-hybrid screen of random HeLa cell cDNAs. Seventy-four clones have been identified which interact with wild-type ER alpha. Three of these clones have been chosen for further characterization.

The first of these, designated hERT A1 (human Estrogen Receptor Trap) encodes a portion of a protein which was originally isolated as a topoisomerase II-binding protein with homology to the cell-cycle regulators Rad4 and Cut5 as well as the DNA repair enzyme XRCC1. High levels of topoisomerase II expression have been shown to correlate with extremely proliferative mammary carcinomas and repression of hormone receptor expression. Topoisomerase II-binding protein may

therefore be involved in ER repression by topoisomerase II in these aggressive tumors. This clone interacts strongly in a ligand-dependent manner with the AF-2 domain of ER and weakly in the hormone-independent manner with the AF-1 domain.

The second clone, designated hERT A2, has sequence homology to a portion of a human 82 kDa acidic protein in the EST database. Similar to hERT A1, the full-length hERT A2 protein interacts strongly in a ligand-dependent manner with the AF-2 domain of ER and weakly in the hormone-independent manner with the AF-1 domain. When overexpressed by transient transfection in a U2-OS osteosarcoma cell line, hERT A2 increases ER-dependent transcriptional activation by at least two-fold in the presence of estradiol.

Another clone that we have shown to interact with ER is the Vitamin D receptor-interacting protein 150 (DRIP150), a subunit of the DRIP coactivator complex. Overexpression of DRIP150 increases ER transcriptional activation. We have focusing our efforts on DRIP150 and its role in ER transcriptional regulation.



Differential regulation of ERα and ERβ by DRIP150.
HeLa cells (1.2 x 10^5 cells/35 mm dish) were transiently transfected using Lipofectamine with paired receptor (0.2 _g/dish) and reporter constructs (0.1 _g/dish) for ERα and ERβ + XETL along with the indicated amount of DRIP150 or empty expression vector to equalize the total amount of DNA per dish and 0.05 _g pCMV-LacZ per dish as an internal control for transfection efficiency. Cells were treated with 10 nM 17_-estradiol (E2) or the ethanol vehicle for 12 h and receptor transcriptional activation was assayed, normalized to β-galactosidase activity and expressed as relative luminescence units (RLU). The average of three independent experiments and standard error is shown.

Key research accomplishments

- Identified DRIP150 as a ER interacting protein
- Demonstrated that DRIP150 increases ER transcriptional activation

Reportable outcomes

None

Conclusion

We have identified DRIP150 as a protein that interacts with the ER and increases ER transcriptional activity when overexpressed. Identification of DRIP150 as a potential ER coactivator may offer a potential new target for turning off ER-dependent transcriptional activation and thereby inhibiting breast cancer growth.